IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
LOCATELLI, Giuseppe et al.
Serial No. 09/831,820 Group Art Unit: 1637
Filed: 06/05/2001 Examiner: FREDMAN, Jeffrey Norman
Attorney docket: 1303-122

For: METHOD FOR THE QUANTITATIVE DETECTION OF NUCLEIC ACIDS

DECLARATION UNDER RULE 132

Honorable Commissioner of Patents and Trademarks
Washington, D.C:

1. I, **Mauro Severo MALNATI,**

Born on November 26, 1962, of Italian nationality,

DECLARE

- 1987: I took a Medical University degree at the University of Genoa;
- 1991: I specialized in Pediatry at the University of Genoa;
- 1988-1989: I was Fellow at the Laboratory of Immunopathology, Department of Clinical Oncology, University of Genoa;
- 1989-1992: I was Visiting Fellow at the Molecular Immunology Section , Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health;
- 1992-1994: I was Visiting Associate at the Molecular Immunology Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health;
- 1995-to date: I have been working as Senior Scientist at the Laboratory of Human Virology, Department of Biotechnology H. San Raffaele;
- 1995 – to date: I have been Principal Advisor for the technical realization of the P2-P3 laboratories, Department of Biotechnology H. San Raffaele;

- 1997-2000: I was Advisor for the development of the TAQMAN/ABI 7700 technology for Science Park Raf
- 1998 – to date: I have been Member of the Scientific Committee for the organization of the "Workshop italiano di PCR Quantitativa"
- I am author or co-author of the following scientific publications:
  - 36 Full Scientific articles published on Scientific journals listed in the Index Medicus
  - 5 book chapters;
  - 87 oral and poster communications to international meetings
- I am co-inventor of US patent application No. 09/831,820

2. In accordance with Examples 1 and 2 of US application No. 09/831,820, I have prepared the following HHV6 Calibrator Probe Sequence: **TACGCAACGCCAACAGACCTAGCGA**

3. I have modified the HHV6 Calibrator Probe Sequence maintaining the same amount of G (5 bases) and C (9 bases) and the same sequence length (25 base pair), according to Gibson et al., Genome Research, vol. 6 no. 10 (1996), to produce the following sequence fragments:

GGGAACTAGAGCCTACTCTCCTCTC (Probe G+C 1)

AGGGACTAGAGCCTACTCTCCTCTC (Probe G+C 2)

4 The sequences of Probe G+C 1 and G+C 2 do not meet the requirements needed to satisfy the TaqMan probe criteria codified in the Primer express software (a dedicated software trademark by ABI corporation) for the following reasons:

i) The presence of a G at the beginning of the probe;

ii) The Melting temperatures (Tm) of both novel sequences are 10 degrees lower than the original HHV-6 Calibrator sequence (Tm=72 °C, sequence G+C 1 Tm=61.5°C, sequence G+C 2 Tm=61.2 °C) and around 1 degree lower than the primers Tms (62 °C). The melting temperatures are calculated with the Nearest Neighbour Algorithms, developed by Breslauer et al. (Predicting DNA duplex stability from the base sequence. PNAS 1986 83: 3746-3750). In the G+C 1 probe the presence of a G at the 5' end will interfere with the Reporter emission. In both probes G+C 1 and 2, a lower Tm will not guarantee an efficient generation of the reporter signal emission. The probe (fluorochrome labelled oligonucleotide) must have a significantly higher Tm (US 520015, column 8: lines 53-68, column 9: lines 1-10, cited in the present US patent application) than the Tm

primers in order to ensure that all the Taq-polimerase molecules engaged in the PCR reaction will find a probe molecule annealed to the target sequence. More specifically (US 5876930 column 9, lines 50-52) the melting temperature of the oligonucleotide probe must be 5-10 degrees higher than the annealing temperature of the PCR reaction. If this condition is satisfied, each amplified DNA molecule generates an active reporter fluorescent signal, and a correlation between PCR amplification and fluorescent detection of the amplified products is possible thus ensuring a correct quantification of the starting material.

5. As illustrated in Figure 1 (Panel A), the Calibrator probe for HHV-6 correctly amplified different amounts (10^1 and 10^5 copies/reaction tested in triplicates) of the starting DNA whereas Probes G+C 1 (Panel B) and G+C 2 (Panel C) failed completely to generate a measurable signal.

6. I further declare that all statements of my own knowledge made herein are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Milan, Italy/17 April 2003
(Place, date)

MALNATI, Mauro Severo
(signature)